

Figure 1:**Amino acid sequences of Cpn60 and Cpn10:****SEQ ID No 1: Cpn10 (encoded by nucleotides pos. 458-751 of Figure 2):**

MKIRPLHDRVRRKEETATAGGILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 2: Cpn60 (encoded by nucleotides pos. 800-2446 of Figure 2):

MAAKDVLFGDSARAKMLVGVNILADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKVASQANDQAGDGTATVLAQAIISEGLKSVAAGMN
PMDLKRIGDKATAAVVAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDELVVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPKAVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

Figure 2:**SEQ ID No 3: DNA coding for Cpn60 and Cpn10:**

Cpn10, pos. 458-751

Cpn60, pos. 800-2446

atcaaaaaatgcagcaaggacagattcctgcccagaattagcagaaggttctttagcactggccggcgcttattattaacgccgg
gtttgtcactgatgcgctgggtttacattactcgtcccgcgacgcgtaaacgttggtccataaggtgattgcattattaccctc
gcatgatgactgcaagcagcttcaagcgacggtagtttcaggaaggctcgttaaatgtacattgcacactgactcgcaaagca
gtcatgaaaaaatcacaattgaaggcgaatataccaagacgataagtaggtatttttcggctagccgttgaaatcctagtaaaagccc

cgataaattaaccatctatttttcacagaggcaatttagcctttgtttacctatttgatcctaacttgggatccaacagttggagaggtctagc
aatgaaaaatccgtccattacatgatcgtattgttgttcgccgtaaagaagaagagaccgcaactgcgggtggtattttttacc
gggcgctgcggcgagaaaaaccaaatcaagggtgtgttatctctgtgggtacttgccgtattcttgataatggttcagtgcaagcgctggc
ggttaacgaaggcgatgttgcgttttttgtaatactcaggtcaaaatactatcgcgatcgcgatggtgaagaattattgattttgaatga
aagtgatctacggcggttttagaagcttaattattacactcacttttttatttaacctacaaafttaaggaaagatcatggctgctaaagacg
tatttttgggtgatagcgcacgcgcaaaaatgttggtagggtgaacatttttagccgacgcagtaagagttaccttaggacctaa
aggtcgtaacgttgttatagaaaaatcatttgggtgcaccgatcatcaccaaagatggtgtttctgttcgcgctgaaatcgaattgaaagaca
aatcgaaaaatcgggcgacagatggttaagggaagttgcttctcaagccaacgaccaagccggtgacggcacaacgacagcgact
gtactagcacaggcgattatcagcgaaggcttgaatctgttgcggctggcatgaatccaatggatcttaaacgtggatttgataaagcta
cggctgctgttgttgcggccattaaagaacaagctcagccttgcgttgatacaaaagcaatcgctcaggtagggacaatctctgccaatg
ccgatgaaacgggttggtcgttaattgctgaagcgatggaaaaagtcggtaaaagaaggtgtgattaccgttgaagaaggcgaaggcctt
gaagacgagcgttgatgtttagaaggcatgcagttcgatcgcgggtacttgtctccgtacttcatcaacaaccaagaaaaatgaccgta
gaaatggaaaatccattaattctatttgggtgataagaaaattgataaccttcaagagctgttgccaattcttgaanaacgtcgtaaatcaggt
cgtccattattgatcgttgcgtgaagatgttgaaggccaagcactagcaacattggtagtaacaacttgcgcggcacattcaagggttgc
agcgggttaaagccccctgttttggcgatcgtcgtaaagcgatgttgcaagatcttgccattcttgacgggtgggtcaggttatttctgaagag
ctagggatgtcttttagaaactcgggatccttcttcttgggtacggcaagcaaggttgtatcgataaaagaaaacaccgtgattgttga
tggcgcgaggtactgaagcaagcgttaatactcgtgttgaccagatccgtgctgaaatcgaaagctcgacttctgattacgacatcga
gttacaagaacgcgttgcctaagcttgcggcgggcggttccgtgattaaggttgggtgcgggttctgaaatggaatgaagagagaa
gaccgtgttgacgatgcacttcatgcaactcgcgcagcgggttgaagaaggtgtgttgcgggtggtggtgtgttgccttgattcgcgcactct
cttcagtaaacggttgttggtgataacgaagatcaaaacgtcggatttgcatgttgacacttctgcgatggaagctcctatccgtcaaatcgc
gggtaacgcaggtgctgaagggtcagtggtgttgataaagtgaatctggcacaggttagccttgggtttaacgccagcacaggtgagt
atggcgatatgattgcgatgggtattttagacctgcataaagtcacgcgttcatctctacaagccgcggcgtctatcgcaggtttgatgat
cacaaccgaagccatggttgcggatgcgcctgttgaagaaggcgctggtggtatgcctgatattgggcggcatgggtggaatgggcg
gtatgcctggcatgatgtaatcacttgtgattcattgtcctgatctgcttaccgtgtaaaaagatcaggctcagggtgctctataaaaaag
ccgtatcttggatgagtggttcttctgctgaaaacgacattcttggagtgcggccttttttgattttgggtcataaaatcagaatattgtgtaatt
ttatgtaactagctggccataatgttgagttcctctgggtggcatgatctcatggtacttcaacttaagcctgattcactgcg
gctttaacagtaaaaaataaacgcaacgtagaacaataaagcgatgpcattaatgaagacgggtgcatttaattcagatc

Figure 3:

SEQ ID No 4: Amino acid sequence of esterase cloned from *Oleispira antarctica* (EstRB8):

EstRB8 (encoded by nucleotides 1145 to 2143 Frame 2 of Figur 4) 333 aa

MKNTLKSSSRFSLKQLGTGALISSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
 SAGDVNLT YMERQGS DKDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
 HGDSEQLLT TDYGLIKQAERLDIFLSGLGVNSFH IAGNSMGG AISAIYSLSHPEKVKSL
 TLIDAAGVDGDT ESEYYK VLAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
 RKT LARAEINN KIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
 VSAAA AFKKIIPQATVHIFPEVGHLP MVEIPSESAKVYEEFLSSIK

Figure 4:

SEQ ID No 5: DNA fragment from plasmid pBK1Est coding for esterase of *Oleispira antarctica* (EstRB8):

Nucleotide positions 1-100 correspond to reverse complement of positions 1196-1121 and 3799-3939 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene).

Positions 101-105 are *Bam*HI – *Sau*3A1 fusion and positions 3795-3798 are *Sau*3A1-*Bam*HI-fusion.

acaggaaacagctatgaccttgattacgccaagctcgaat taacccctcactaaagggaacaaaagctggagctcgcgcgcctgcag
 gtcgacactagtggtcaacggcggtcatggtactggctgagtcagcgcataatgccgatgcgatactggccgtcatgactgagtact
 tcttctgctagcaccgatttttctaatagcgcagcttcttttctgaacgggcaactgatgtagttttttactaacggcgttttagcatgg
 taaactcttgatattcaaaattattactgttcattacaatcatagtagcaggctagaggcccaaaattgcagctgatattcacctttattatc
 taagcattattacactcatcgcggtgttattattgtgctaaataaaatacccgtagcggaaaaattcagcaaatagccaaagaaaacga
 ttggcaataccaagaattcatcgatttgatgatgacattaagcaggcaaaacttggcctattaaactacagtcaaaatgcaatttttagacat
 ctcatccaagcaactgacgaacactatggcttagcgttaagaccttgactgtcagcgttagaaccttcaggtattcacaatagcagctt
 tattttttaccctcgcactaaagactgaattcaataacctacacatttgcttaagtcgacatattcaagataaagatgccttcactgacatca
 gtcaccaacaatcaatcaaacaccaataccaatcgcaaaaactcataaaactagccgatcaccaaatccaaaagcgttcaaaaatgaa
 acgagcacgtcacacaaaatcaattatacgctaacgaaccagggtcaaaacttatcgttttttgagcacggttgttccactaatgaaagaga
 aaagtcgttaattcactggctttggcgtatccgcaccttcacatagaaattagtaattggcatgctactggcctttaaaaagaatcagttaatt
 gaagaaacctcgttatctcagccattaccgctgtagccgaatttgcgcttatcctcagccatgattaaactgacgccaattaatataagac
 atactaatataaactcccttaattgagaagaataatgaaaaacacactcaaatcctcatcacgttttagctgaaacaactcggcaccggc
 gctctgattatctccagttgttcttcgggtggtgcaccacaacacaagataattatacacaggggttatgtctcttcgagagacagc
 gctggcctagaagttaaaacagcctctgccggtgacgtcaatcttacttatatggaacgccaaggcagtgacaaaagataatgccgaaag

cggtattttattacacggtttctctgctgataaagataactggattcttttaccaaagaattc gatgaaaaatcatgttatcgtctgctgattta
gcggggacatggcgattcagaacaattattaacgactgattacgggtctcataaaacaagccgagcgttagatatcttctatctggttagg
ggtaacacatttcacatcgccggtaattcaatggggggggctatcagcgcgaatctacagtttgagtcacccagagaaaagttaaaagtctt
acattgatcgatgcagcagggtgctgatggcgatactgaaagcgaatactacaaagtttggcagaaggtaagaatcctttaattgcaact
gatgaagcaagttttgaataccgcatgggtttcacatgactcagcctccttctaccttggccactaagaccttctttattacgtaaaacg
ctagcccggtgccgagatcaataacaaaatttttccgatatgcigaaaaccaagaacgtttaggaatgactaactttcaacagaaaattg
aagtgaanaatggctcaacatccattgccaaactgattatgtggggcaagaagatcgcgttcttgacgtatccgcagcagcgcccttc
aaaaaataattccacaagcaactgttcatatttttctgaagtaggccactacctaigttagaaattcctagtgaagcgtctaaagtattat
gaagagttttgtcctctattaaataagagcacataatcatgactgactataaacagccaagcatttaaaatgcttggctgttttatttaattg
ccaaattattcaacgaccaagctctgcggtaaaatcgcagtggttctgttttcatcaacagcaacaaacgtgaataacccccgtaatcg
catttttctgattatcaaaaatacatactttccaccagcatattaaactcaacttttaactcgtccgccctacctctataacactggcagtcatt
cgacaatggtacctgcgggaacaggatgcttaaaatcgattcgatcactgctgacggttacgatgctttgtcgagaaaaacgagtcgct
gcaataaaagaaacctcatccatccactgcattgcagtgccaccgaataacgtatcatgatgatttgtgtctctgaaataaccgctttaga
aatagtggttttgatagcgctttcgctgcgcaataatcttctctgtaagagttgcggatggcatacataaactcgcttgattaagatta
ataataaatagttaacagtatattgaactgagggtctgaagaactctaatacctctgaagaactttgaggccgctagagagaaaagacca
gtgataataattcatcttgccatgagagcttatcatgaaagcctgtgcttaaaatcaatcattatatttcatctttaattgaaataataccaat
atatttcataataatttcacactaccccttatctcactagacttcccgcgcataggcgcaacaatcaacgcaagttcacataaagcgggttc
gctgcaacacatgccctagcgtctaaagtagcacgcacaactggccagtcgtactagccctttgcgattcgtgcagacgagcaac
aagcgcgtattaaacttacctaatttctaaccaccaccattggttctttccacaaactcaaaaaactcgtcaaatccgcttgcaatttaaacg
cgatgacatagatctaatacgattatcaaaacccgattcaagcgtcattaaaaacgcaccactggcaagaagtctacctgcactgacca
atatgcaagcggcgccggaagagctgcccttgatcgatcaagaagaaggagcagcaaaagaggaaaacaatcaaaaagaggaga
gcaatcaataaaaaacgagttattgaggatttaatttaaaacaggtatattaataccctctctcgtagtaaacatgactgtattacacaa
aaataaatagagggtataccatgtcaaacatctggttgaaagtaccaagattgaagtattaaaccgtcaaatggaaaatactgcctgcagc
aacttaggcattcaaatcacagaaattggcgatgattatatactggcacaatgccagcagatgcacgtacctccagccaatgggactg
attcatggcgggtcaaatgtattgctggcagaaacactgggcagcatggcagctaactgctgtattaatgtctcaagaatattgtgttg
ccaagaaattaacgccaaccacatacgcggtgttcgttcggcatagtgactggcacagcaacgctagtacacaaaggagaacctc
ccagatttgggaaattcgcacgttaacgatccaagaattcaaaaagcttctcgagagtacttctagagcggccggggcccatcgatt
ttccacccgggtgggtaccaggttaagtgtaccaattcgcctatagtgagtcgtattacaattcactggccgtcgttttac

Figure 5:

Amino acid sequences expressed from vector pBK1CpnEst: - the co-expression of fragments encoding native chaperonins with the esterase gene (EstRB8), all from *Oleispira antarctica*

SEQ ID No 6: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 6) 97 aa:

MKIRPLHDRVVRKEEETATAGGILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVF GKYSQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 7: cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 6) 548 aa:

MAAKDVLFGDSARAKMLVGVNLAADVRLTGPVKGRNVVIEKSFGAPIITKDGVS
AREIELKDKFENMGAQMVKEVASQANDQAGDGTATVLAQAIISEGLKSVAAGMN
PMDLKRIGDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADETVGRLIAEAMEKV
GKEGVITVEEGKGLEDEL DVVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTTVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAEGSVVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

SEQ ID No 8: estRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 6) 333 aa:

MKNTLKSSSRFSLKQLGTGALISSLFFGGCTTTQQDNLYTGVM SLARDSAGLEVKTA
SAGDVNLTYMERQGSDDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLTDDYGLIKQAERLDIFLSGLGVNSFHIA GNSMGGAISAIYSLSHPEKVKSL
TLIDAAAGVDGDTSEYYKVLAE GKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL
RKTLARAEINNKFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
VSAAAFAFKKIIPQATVHIFPEVGHLP MVEIPSESAKVYEEFLSSIK

Figure 6:

SEQ ID No 9: pBK1CpnEst: - the fusion of native chaperonine-coding fragments with
esterase of *Oleispira antarctica* (EstRB8)

The DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SalI* site (encoded by pos. 2138-2143 of Figure 7):

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

Small letters – the Cpn10-Cpn60 encoding fragment,

Capital italics – fragments of vector pBK-CMV

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

*ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCCCTCACTAAAGGGA
ACAAAAGCTGGAGCTC*taataacttgggatccaacagttggagagtctagcaaatgaaatccgtccattacatgatcgtatt
gttgttcgccgtaaagaagaagagaccgcaactgcgggtggtattatttaccggcgctgcggcagaaaaaccaaatacaggtgttgt
tatctctgtgggtactggccgtattcttgataatggttcagtgcaagcgctggcggttaacgaaggcgtgtgtcgttttggtaaatactc
aggtcaaaatactatcgatcgtatggtgaagaattattgattttgaatgaaagtatactacggcggtttagaagcttaattattacactca
ctttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacgtattatttggatagcgcacgcgcaaaaatgttggttaggt
gtaaacatttttagccgacgcagtaagagttaccttaggacctaaggtcgtaacgttgttatagaaaaatcatttgggtgcaccgatcatcac
caaagatgggtgtttctgttgcgcgtgaaatcgaattgaaagacaaatcgaaaaatgggcgacagatggttaaggagttgcttctca
agccaacgaccaagccggtgacggcacaacgacagcgactgtactagcacaggcgattatcagcgaaggcttgaaatctgttgcgg
ctggcatgaatccaatggatcttaaacgttggtattgataaagctacggctgctgttggccgccaataaagaacaagctcagccttgctg
gatacaaaagcaatcgctcaggtagggacaatctctgccaatgccgatgaaacggttggtcgtttaattgctgaagcgtgaaaaagt
cggtaaagaaggtgtgattaccgttgaagaaggcaaaggccttgaagacgagcttgatgtttagaaggcatgcagttcgatcgcggtt
acttgtctccgtacttcatcaacaaccaagaaaaatgaccgtagaatggaaaatccattaatctattggttgataagaaaattgataac
cttcaagagctgttgccaattctgaaaacgtcgctaaatcaggtcgtccattattgatcgttgctgaagatgttgaaggccaagcactagc
aacattggttagtaacaacttgcgcggcacattcaaggttgacgcggttaagcccctggttttggcgatcgtcgtaaagcgtatgttga
agatcttggccatcttgacgggtggtcaggttattctgaagagctagggatgtctttagaaactcgggatccttcttcttgggtacggcaa
gcaaggttggtatcgataaagaaaacaccgtgattgttgatggcgaggtactgaagcaagcgttaatactcgtgttgaccagatccgtg
ctgaaatcgaaagctcgactctgattacgacatcgaaaagttacaagaacgcgttgctaaagcttgcggcggttgccgtgattaag

7/15

AATCGCAGTGGGTTTCTTGTTTTTCATCAACAGCAACAAACGTGAAATACCCCGTA
ATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACCTTCAAC
TTTTAAACTCGTCCGCCCTACCTCTATAAACTGGCAGTCAATTCGACAATGGTAC
CTGCGGGAACAGGATGCTTAAAATCGATTGATCACTGCTGACGGTTACGATGCT
TTGTGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTGCATT
GCAGTGCCACCGAATAACGTATCATGATGATTTGTTGTCTCTGGAAATACCGCTTT
AGAAATAGTGGTTTTTGATACGCGCTTTGCTGCGCAATAATATCTTCTCTGCTAA
GAGTTGCGGATGGCATACTAACTCGCTTGATTAAGATTAATAATAAATAGTTA
ACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACTTTGAG
GCCGCTAGAGAGAAAAAGACCAGTGATAATATTTTCATCTTGCCATGAGAGCTTATC
ATGAAAGCCTGTGCTTAAAATCAATCATTATATTTATTTCATCTTTAATTGAAATAA
TACCAATATATTTTCATATATAATTTTCACTACCCTTATCTCACTAGACTTCCCGC
GCATAGGCGCAACAATCAACGCAAGTTCACAATAAAGCGGTTGCTGCAACAC
ATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGCCCCTT
TGCGATTGCTGCAGACGAGCAACAAGCGCTATTAACTTACCTAAATTTCTAACC
ACCACCATTGGTTCTTTTCCACAACTCAAAAACTCGTCAAATCCGCTTGCAATT
TAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGCTCATT
AAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAGCGGC
GGCGGAAGAGCTGCCTTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGGAAA
ACAATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTTAAT
TTTAAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTACAC
AAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAGATTG
AAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTCAAAT
TACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACGTACC
TTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAAACAC
TGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTGTTGG
CCAAGAAATTAACGCCAACCACATACGCGGTGTTTCGTTCCGGCATAGTGACTGGC
ACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTTCGCATC
GTTAACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCGCGGG
CCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTGCCCCTATAGT
GAGTCGTATTACAATTCACTGGCCGTCGTTTTAC

Figure 7:

Amino acid sequences expressed from vector pBK1CpnSREst: - the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala::est)

SEQ ID No 10: cpn10 (nucleotides 113 to 403: Frame 2 of Figure 8) 97 aa:

MKIRPLHDRVVRKEETATAGGILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSGQNTIDIDGEELLILNESDIYGVLEA

Below – **Capital bold letters** are the mutations introduced

SEQ ID No 11: stabilized single ring mutant of cpn60 (nucleotides 455 to 2098: Frame 2 of Figure 8) 548 aa:

MAAKDVLFGDSARAKMLVGVN~~L~~ADAVRVTLGPKGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGT~~T~~TATVLAQAIISEGLKSVAAGMN
PMDLKR~~G~~IDKATAAVVAAI**K**EQAQPCLDTKAIAQVGTISANADET~~V~~GRLIAEAMEKV
GKEGVITVEEGKGLEDEL~~D~~VVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLV**D**KK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVVNNLRGTFKVA**A**AVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTVVGDNEDQNVGIALALRAMEAPIRQI
AGNAGAA**A**G**A**AVVDKVKSGTGSFGFNASTGEYGDMIAMGILDPKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGGMPGMM

SEQ ID No 12: EstRB8 (nucleotides 2579 to 3577: Frame 2 of Figure 8) 333 aa:

MKNTLKSSSRFSLKQLGTGALIISLFFGGCTTTQQDNLYTGVM**S**LARDSAGLEV**K**TA
SAGDVNLTYMERQGS**D**KDNAESVILLHGFSADKDNWILFTKEFDEKYHVIAVDLAG
HGDSEQLLTDDYGLIKQAERLDIFLSGLGVNSFH**I**AGNSMGGAISAIYSLSHPEKV**K**SL
TLIDAAGVDGDTESEYYK**V**LAEGKNPLIATDEASFEYRMGFTMTQPPFLPWPLRPSLL

RKTLARAEINNKIFSDMLKTKERLGMTNFQQKIEVKMAQHPLPTLIMWGKEDRVLD
 VSAAAFKKIIPQATVHIFPEVGHLPVMEIPSESAKVYEEFLSSIK

Figure 8:

SEQ ID No 13: DNA sequence of vector pBK1CpnSREst: the expression cassette for the co-expression of the stabilized single ring mutant chaperonin with the esterase gene (EstRB8) from *Oleispira antarctica* (cpn10::stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala::est)

Nucleotide positions 1-75 correspond to reverse complement of positions 1196-1121 and positions 5233-5273 correspond to reverse complement of 1043-952 of pBK-CMV vector (Stratagene)

DNA fragment coding for Cpn10 and Cpn60 is flanked by *SacI* site (pos. 69-75) and *SaII* site (pos. 2138-2143).

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Capital italics – fragments of vector

Capital letters – fragment coding for EstRB8 from plasmid pBK1Est

Capital bold letters = introduced mutations

ACAGGAAACAGCTATGACCTTGATTACGCCAAGCTCGAAATTAACCTCACTAAAGGGA
*ACAAAAGCTGGAGCTC*ctaatacttgggatccaacagttggagagtctagcaaatgaaaatccgtccattacatgatcgtatt
 gttgttcgccgtaaagaagaagagaccgcaactgcgggtggtattatttaccggcgctgcggcagaaaaacaaatcaaggtgttgt
 tatctctgtgggtactggccgtattcttgataatggttcagtgcaggcgctggcggttaacgaaggcgatgtgtcgttttggtaaatactc
 aggtcaaaatactatcgatcgcgtggaagaattattgatttgaatgaaagtatactacggcgttttagaagcctaattattacactca
 cttttttatttaacctacaaaatttaaggaaagatcatggctgctaaagacgtattatttggatagcgcacgcgcaaaaatgttgtaggt
 gtaaacatttttagccgacgcagtaagagttaccttaggacctaaaggctgtaacgttggttatagaaaaatcatttggtgcaccgatcatcac
 caaagatggtgtttctgttgcgcgtgaaatgaattgaaagacaaatcgaaaacatggcgccacagatggttaaggagttgcttctca
 agccaacgaccaagccggtgacggcacaacgacagcgactgtactagcacaggcgattatcagcgaaggcgttgaatctgttcggg
 ctggcatgaatccaatggatcttaaacgtggtattgataaagctacggctgctgttggtccgccattaaagaacaagctcagccttgcttg

11/15

CCTTTCCTACCTTGGCCACTAAGACCTTCTTTATTACGTAAAACGCTAGCCCGTGC
CGAGATCAATAACAAAATTTTTCCGATATGCTGAAAACCAAAGAACGTTTAGGA
ATGACTAACTTTCAACAGAAAATTGAAGTGAAGTGGCTCAACATCCATTGCCAA
CACTGATTATGTGGGGCAAAGAAGATCGCGTTCTTGACGTATCCGCAGCAGCGGC
CTTCAAAAAATAATTCCACAAGCAACTGTTTCATATTTTTCTGAAGTAGGCCAC
CTACCTATGGTAGAAATTCCTAGTGAAAGCGCTAAAGTTTATGAAGAGTTTTTGT
CCTCTATTAAATAAGAGCACATAATCATGACTGACTTATAAACAGCCAAGCATTT
AAAATGCTTGGCTGTTTATTTAATGGCCAAATTATTCAACGACCAAGCTCTGCG
GTAAATCGCAGTGGGTTTCTTGTTTTTCATCAACAGCAACAAACGTGAAATACCC
CGTAATCGCATTTTTCTGATTATCAAAATACATACTTTCCACCAGCATATTAACCT
CAACTTTTAAACTCGTCCGCCCTACCTCTATAACACTGGCAGTCAATTCGACAATG
GTACCTGCGGGAACAGGATGCTTAAAATCGATTTCGATCACTGCTGACGGTTACGA
TGCTTTGTGCGAGAAAAACGAGTCGCTGCAATAAAAGAAACCTCATCCATCCACTG
CATTGCAGTGCCACCGAATAACGTATCATGATGATTTGTTGTCTCTGGAAATACC
GCTTTAGAAATAGTGGTTTTTGATACGCGCTTCGCTGCGCAATAATATCTTCTCT
GCTAAGAGTTGCGGATGGCATAACATAAACTCGCTTGATTAAGATTAATAATAAAT
AGTTAACAGTATATTGAACTGAGGGTCTGAAGAACTCTAATACCTCTGAAGAACT
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AATAATACCAATATATTTTCATATATAATTTACACTACCCCTATCTCACTAGACTT
CCCGCGCATAGGCGCAAACAATCAACGCAAGTTCACAATAAAGCGGTTTCGCTGC
AACACATGCCCTAGCGTCTAAAGTAGCACGCACAACACTGGCCAGTCGTACTAGC
CCCTTTGCGATTTCGTGCAGACGAGCAACAAGCGCTATTAACTTACCTAAATTC
TAACCACCACCATTTGGTTCTTTTCCACAACTCAAAAACTCGTCAAATCCGCTTG
CAATTTAAACGCGATGACATAGATCTAATCGATTATCAAACCCGCATTCAAGCGC
TCATTAAAAACGCACCACTGGCAAGAAGTTCTACCTGCACTGACCAATATGCAAG
CGGCGGCGGAAGAGCTGCCTTTGATCGATCAAGAAGAAGGGAGCAGCAAAGAGG
AAAACAATCAAAAAGAGGAGAGCAATCAAATAAAAACGAGTTATTGAGGATTTT
AATTTTAAACAGGTATATTAATACCCTCTCTCGTAGTAAACAATGACTGTATTTA
CACAAAAATAAATAGAGGTATACCATGTCAAACATCTGGTTTGAAGTACCAAAG
ATTGAAGTATTAAACCGTCAAATGGAAAATACTGCCTGCAGCAACTTAGGCATTC
AAATTACAGAAATTGGCGATGATTATATCACTGGCACAATGCCAGCAGATGCACG
TACCTTCCAGCCAATGGGACTGATTCATGGCGGCTCAAATGTATTGCTGGCAGAA
ACACTGGGCAGCATGGCAGCTAACTGCTGTATTAATTTGTCTCAAGAATATTGTG

TTGGCCAAGAAATTAACGCCAACACATACGCGGTGTTTCGTTCCGGCATAGTGAC
TGGCACAGCAACGCTAGTACACAAAGGAAGAACCTCCCAGATTTGGGAAATTTCG
CATCGTTAACGATCCAAAGAATTCAAAAAGCTTCTCGAGAGTACTTCTAGAGCGGCCG
CGGGCCCATCGATTTTCCACCCGGGTGGGGTACCAGGTAAGTGTACCCAATTTCGCCCT
ATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTAC

Figure 9:

Amino acid sequence of the stabilized single ring mutant Glu461Ala/Ser463Ala/Val464Ala of Cpn60:

SEQ ID No 14: Cpn10 (nucleotides 458-751 of Figure 10):

MKIRPLHDRVRRKEETATAGGILPGAAAEKPNQGVVISVGTGRILDNGSVQALA
VNEGDVVVFGKYSQNTIDIDGEELLILNESDIYGVLEA

SEQ ID No 15: Cpn60 (nucleotides 458-751 of Figure 10):

MAAKDVLFGDSARAKMLVGVNLADEVRTLGPVGRNVVIEKSFGAPIITKDGVSV
AREIELKDKFENMGAQMVKEVASQANDQAGDGTATVLAQAIISEGLKSVAAGMN
PMDLKRIGDKATAAVVAAIKEQAQPCLDTKAIAQVGTISANADEVGRLLIAEAMEKV
GKEGVITVEEGKGLEDELVDVEGMQFDRGYLSPYFINNQEKMTVEMENPLILLVDKK
IDNLQELLPILENVAKSGRPLLIVAEDVEGQALATLVNLRGTGFKVAAVKAPGFGD
RRKAMLQDLAILTGGQVISEELGMSLETADPSSLGTASKVVIDKENTVIVDGAGTEAS
VNTRVDQIRAEIESSTSDYDIEKLQERVAKLAGGVAVIKVGAGSEMEMKEKKDRVD
DALHATRAAVEEGVVAGGGVALIRALSSVTTVVDNEDQNVGIALALRAMEAPIRQI
AGNAGAAGAAVVDKVKSGTGSFGFNASTGEYGDAMIAMGILDPAKVTRSSLQAAASI
AGLMITTEAMVADAPVEEGAGGMPDMGGMGGMGMPGMM

Figure 10:SEQ ID No 16: DNA sequence of the stabilized single ring mutantGlu461Ala/Ser463Ala/Val464Ala:

In the DNA sequence:

Small letters – the Cpn10-Cpn60 coding fragment,

Big bold letters = introduced mutations

atcaaaaaatgcagcaaggacagattcctgcccagaattagcagaagggttctttagcactggccggcgctttattataacgccgg
 gttttgtcactgatgcgctgggtttacattactgtccccgcgacgcgttaaagcgttggtccataagggtattgcattattaccctc
 gcatgatgactgcaagcagcttcaagcgacgggtagtttcagggaaggctcgtttaaagatgtacattcgacactgactcgcaagca
 gtcataaaaaatcacaattgaaggcgaatatacacaagacgataagtaggtatttttcggctagccgttgaatcctagtaaaagccc
 cgataaattaaccatctattttcacagaggcaatttagccttggttacctattgatcctaatacttgggatccaacagtggagagtctagc
 aatatgaaaatccgtccattacatgatcgtattgttgcgcgttaaagaagaagagaccgcaactgcgggtggtattatttacc
 gggcgctgcggcgagaaaaaccaatcaaggtgttattctctgtgggtactggccgtattcttgataatggttcagtgcgaagcgctggc
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 aagtgatctacggcgtttgaagcttaattattacactcacittttatttaacctacaaaatttaaggaaagatcatggctgctaaagacg
 tatttttgggtgatagcgcacgcgcaaaaatgttggtaggtgtaaacatttttagccgacgcagtaagagtaccttaggacctaa
 aggtcgtaacgttgttatagaaaaatcatttgggtgcaccgatcatcaccaaagatggtgttctgttcgcgtgaaatcgaattgaaagaca
 aattcgaaaaatcggcgacagatggttaagggaaggtgttctcaagccaacgaccaagccgggtgacggcacacgacagcgact
 gtactagcacaggcgattatcagcgaaggcttgaatctgttgcggctggcatgaatccaatggatcttaaacgtgttattgataaagcta
 cggctgctgttgttccgccattaaagaacaagctcagccttgccttgatacaaaagcaatcgtcaggtaggagacaatctctgc caatg
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 gaccgtgttgacgatgcacttcatgcaactcgcgcagcgggtgaagaaggtgttgcgggtggtgttgccttgattcgcgcactct
 cttcagtaaccgttgttgggtgataacgaagatcaaacgcgttgattgacattgcacitcgtgcgatggaagctcctatccgtcaaatcgc

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agccgtatctttgatgagtgttcttctgctgaaaacgacattcttgagtgcggttttttgatttggtcataaaattcagaatattgtga
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gctttaacagtaaaataataacgcaacgtagaaacataataagcgtatggcattaatgaagacggctgcatttaattcagatc